

REC'D 10 OCT 2001



PCT

WIPO

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| | | |
|---|---|---|
| Applicant's or agent's file reference THE 31018 WO | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
| International application No. PCT/US00/20674 | International filing date (day/month/year) 27/07/2000 | Priority date (day/month/year) 27/07/1999 |
| International Patent Classification (IPC) or national classification and IPC C12N9/00 | | |
| Applicant THE SALK INSTITUTE FOR BIOLOGICAL STUDIES et al. | | |
| <p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 9 sheets, including this cover sheet.</p> <p><input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of sheets.</p> | | |
| <p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none">I <input checked="" type="checkbox"/> Basis of the reportII <input type="checkbox"/> PriorityIII <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicabilityIV <input type="checkbox"/> Lack of unity of inventionV <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statementVI <input type="checkbox"/> Certain documents citedVII <input type="checkbox"/> Certain defects in the international applicationVIII <input checked="" type="checkbox"/> Certain observations on the international application | | |
| Date of submission of the demand 16/02/2001 | Date of completion of this report 05.10.2001 | |
| Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 | Authorized officer Vix, O Telephone No. +49 89 2399 7326  | |

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/20674

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-193 as originally filed

Claims, No.:

1-39 as originally filed

Drawings, sheets:

1/15-15/15 as originally filed

Sequence listing part of the description, pages:

1-4, filed with the letter of 11.01.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/US00/20674

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 38-39.

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 38-39.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/20674

1. Statement

| | | | |
|-------------------------------|------|--------|---------------------------|
| Novelty (N) | Yes: | Claims | 3-5,9-16,19-21,25,28-37 |
| | No: | Claims | 1,2,6-8,17-18,22-24,26-27 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-37 |
| Industrial applicability (IA) | Yes: | Claims | 1-37 |
| | No: | Claims | |

2. Citations and explanations see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/US00/20674

Reference is made to the following documents:

- D1: JEZ J M ET AL.: 'Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis.' FASEB JOURNAL. MEETING INFO.: ANNUAL MEETING OF THE AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY ON BIOCHEMISTRY AND MOLECULAR BIOLOGY 99 SAN FRANCISCO, CALIFORNIA, USA MAY 16-20, 1999, vol. 13, no. 7, 23 April 1999 (1999-04-23), page A1392
- D2: DATABASE PDB [Online] ID 1BI5, 22 June 1999 (1999-06-22) FERRER J-L ET AL.: 'Chalcone synthase from Alfalfa' cited in the application
- D3: JUNGHANS H ET AL.: 'Stress responses in alfalfa (*Medicago sativa* L.). 15. Characterization and expression patterns of members of a subset of the chalcone synthase multigene family' PLANT. MOL. BIOL., vol. 22, no. 2, May 1993 (1993-05), pages 239-253, cited in the application
- D4: TROPF S ET AL.: 'Reaction mechanisms of homodimeric plant polyketide synthases (stilbene and chalcone synthase) - a single active site for the condensing reaction is sufficient for synthesis of stilbenes, chalcones, and 6'-deoxychalcones' JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 14, 7 April 1995 (1995-04-07), pages 7922-7928,
- D5: PREISIG-MÜLLER R ET AL.: 'Plant polyketide synthases leading to stilbenoids have a domain catalyzing malonyl-CoA:CO₂ exchange, malonyl-CoA decarboxylation, and covalent enzyme modification and a site for chain lengthening' BIOCHEMISTRY, vol. 36, no. 27, 8 July 1997 (1997-07-08), pages 8349-8358,
- D6: RAIBER S ET AL.: 'Molecular and enzymatic characterization of two stilbene synthases from Eastern white pine (*Pinus strobus*). A single Arg/His difference determines the activity and the pH dependence of the enzymes.' FEBS LETT., vol. 361, no. 2-3, 20 March 1995 (1995-03-20), pages 299-302,
- D7: HELARIUTTA Y ET AL.: 'Chalcone synthase-like genes active during corolla development are differentially expressed and encode enzymes with different catalytic properties in *Gerbera hybrida* (Asteraceae)' PLANT MOLECULAR BIOLOGY, vol. 28, no. 1, April 1995 (1995-04), pages 47-60
- D8: ECKERMANN S ET AL.: 'New pathway to polyketides in plants' NATURE, vol. 396, 26 November 1998 (1998-11-26), pages 387-390, cited in the application
- D9: SCHRÖDER J: 'A family of plant-specific polyketide synthases' TRENDS IN PLANT SCIENCE, vol. 2, 1997, pages 373-378,

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Novelty (Art. 33(2) PCT)

1.1 Claim 1 refers to an isolated polyketide synthase (PKS) comprising an active site characterised by the spatial coordinates of critical amino-acids alpha-carbons. As such, this claim has to be considered as a **product claim** (a specific enzyme in the present case) which can be unambiguously characterised by its primary sequence. The further enzyme characterisation using the spatial coordinates of 14 active site alpha carbons (new parameter definition of a known enzyme) does not render the product novel if said enzyme was already purified and characterised in the prior art. Furthermore, the man skilled in the art assumes that the correctly folded and biologically active PKS enzyme of the prior art intrinsically possesses the same active site three-dimensional structure as the one described in the application.

Consequently, independant claims 1 and 7 are considered to lack novelty in view of the known PKS purified in the prior art (e.g. in D3 and D4). It is also to be noticed that the chalcone synthase (CHS) crystal structure discussed in D1 (CHS structure also in complex with resveratol) and disclosed in D2 (PDB accession number 1BI5) has been public before the priority date of the present application. Thus, D1-D2 relating to CHS structure are considered prejudicial to the novelty of claim 1, 6-7 and claims relative to CHS (i.e. claims 17, 22-24, 26-27).

It is to be noticed that the above comments could lead to a unity problem (depending what is considered as the common inventive concept between all claims).

1.2 D4 and D5 disclose active site mutants of two different PKS (Stilbene and chalcone synthase: STS and CHS). According the Fig 2 numbering, the mutant C169S in D4 -for CHS or STS- corresponds to the C164A mutant of claim 2. In D5, the STS mutant C164A in Table 1 also falls within the scope of claim 2 (PKS with Ala or Ser at position 164). Thus claim 2 and the corresponding claims 8 and 18 (see item VIII) lack novelty in view of D4 and/or D5.

- 1.3 In view of the available prior art, the PKS of claims 3-5 appear to be variants of wild type CHS. However, there is no clear consensus numbering specified for the claimed PKS within a enzyme family showing a high identity level (over 65% identity according to D8). Considering the CHS sequence numbering, the PKS of claims 3-5 and their specific mutation points (at position 303, 336, or 215) appear to be novel over the available prior art. The same reasoning applies for their nucleic acid encoding sequences mentioned in claims 9-11.

2. Inventive step (Art. 33(3) PCT)

- 2.1. Claims 3-6 relate to PKS mutants. The problem to be solved by the present invention may therefore be regarded as the provision of PKS mutants.

Many different polyketide synthase such as stilbene or chalcone synthase (STS or CHS) were known (see D3-D9) and their mechanisms of action have been characterised (e.g. D4-D6).

In absence of evidence showing unknown or unexpected effects or properties of presently claimed PKS and their mutants (claims 3-6), polynucleotides encoding them (claims 9-11) and "crystalline forms" or "crystalline complex" thereof (claims 19-27, see item VIII), the presence of an inventive step cannot be acknowledged since the mere provision of mutants of known PKS (such as CHS or STS), using methods which are known in the art for this purpose, does not appear to involve an inventive activity to a person skilled in the art. Thus, claims 3-6, 9-11 and 19-27 do not meet the requirements of Art. 33(3) PCT.

- 2.2 Claims 12 relates to a method of predicting the activity and/or substrate specificity of a "putative" PKS. Said claim only describe a general molecular structure comparison method between two structures. There is no technical features allowing this "prediction" of the activity and/or substrate specificity of a "putative" PKS based on the structural information. Furthermore, there is no indications in claims 12-16 which molecule falls within the scope of these "putative" PKSs and what has to be interpreted by the term "representation of a known polyketide synthase". Thus said claims are ambiguous and open to interpretation (see item VIII).

Moreover, claim 12 defines a desideratum (the "prediction") that lacks the technical features that would allow the skilled person to realise said wish.

In absence of any surprising effect derived from a clear technical teaching, claims 12-16 appear to lack an inventive step. Thus said claims do not meet the requirements of Article 33(3) PCT.

- 2.3 The same observation as in 2.2 applies for the claims 28-32 which relate to methods for identifying a potential substrate of a PKS or with claims 33-37 dealing with methods for identifying a potential inhibitor of PKS. These claims include general features in the field of genetic engineering and/or structure based drug design. In absence of clear distinguishing features and technical teaching, it would have been obvious to a person skilled in the art to arrive at the claimed subject-matter based on common technical knowledge and routine methods in the fields of rational drug design (enzyme/ligand structure-function analysis using concepts such as in LUDI or DOCK programs) or protein engineering.

In absence of any surprising technical effect, claims 28-37 appear to lack an inventive step. Thus these claims do not meet the requirements of Article 33(3) PCT.

Re Item VIII

Certain observations on the international application

With regard to the present set of claims, the following general remarks are made:

Article 6 PCT requires amongst other things that the claims, which define the matter for which protection is sought, be clear. This has to be interpreted as meaning not only that a claim must be comprehensible from the technical point of view, but also that it must define clearly the object of the invention(i.e. contain all the essential features thereof). These essential features are regarded as all the features necessary to obtain the desired effect, or to solve the technical problem with which the application is concerned. The technical features should enable the person skilled in the art to perform the invention over the whole area of the claimed invention without undue burden, that is without experimentation or application of inventive skill.

1. The term "representation" used in claim 12 lacks clarity and is open to interpretation.

- 2 Concerning the subject-matter of claims 17-27, it is not clear what falls under the term "crystalline form of the polyketide synthase" or "crystalline complex". Two different interpretations lead to different observations:
- a. The "crystalline" forms or complexes of the PKS cited in claims 17-22 and 23-27 (in presence of a substrate) can be interpreted as pure and homogeneous enzymes in solution - in a crystal the proteins are only held together by a multiplicity of bonding interactions (hydrogen, ionic... interactions) which does not allow them to be distinguished from the known product in the prior art. Thus, if the product (purified enzyme) is known from the prior art, the novelty of said claims can not be acknowledged.
 - b. The "crystalline" forms or complexes cited in claims 17-27 could also refer to the set of coordinates of said enzymes determined by X-ray crystallography (it would correspond to a coordinate listing). Such structure data is not regarded as patentable invention within the meaning of Rule 67 (v) PCT since it relates to a presentation of information (protein model structure coordinates) stored on a computer.